iGEM TU/e 2014
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2014.igem.org/Team:TU_Eindhoven

Protocol bacteria culturing for microfluidics

This protocol is for culturing fluorescent bacteria to use them in a microfluidics device.

Technische Universiteit
Eindhoven
University of Technology

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Bacteria culturing for microfluidics

1 Growth of small bacterial cultures:

- Fill 12 mL sterile culture tubes with 5 mL LB. Work near the Bunsen burner flame
- Add 5 µL kanamycin (30 µg/mL) to the cultures
- Pick cells from the glycerol stock using a sterile pipette tip and eject the pipette tip into the culture tube
- Grow the bacteria overnight at 37°C and 250 rpm

2 Protein expression:

- Transfer 100 µL of the small culture to new 10 mL LB culture **(with 10 µL kanamycin (30 µg/mL))**
- Grow the bacteria at 37 °C and 250 rpm
- Measure OD, a cell division cycle takes ~20 minutes. OD measurement first requires blank measurement with LB
- When OD = 0.6 continue with next step.
- **For a 5 mL culture**: add 5.62 µL IPTG from the 1M stock.
- Perform protein expression for ~15 h at 25 °C and 250 rpm.
- Spin down the cells for 15 min at 3,000 xg.
- Discard supernatant.
- Add 1 ml PBS and transfer to 1,5 mL Eppendorf microcentrifuge tube.
- Spin down the cells for 1 min at 13,000 xg.
- Discard supernatant
- Add 1 ml PBS
- Fill 1 OD cuvette with 1 mL dH2O for blank OD measurement.
- Fill 1 OD cuvette with 950 µL dH2O and 50 µL of culture sample (dilution of 20x)
- Measure OD, this has to be lower than 1, otherwise make a higher dilution.
- Calculate OD of culture sample. (If you made a dilution of 20X then the OD of the culture is 20X the OD of the dilution.
- Calculate amount of cells in culture.